REMARKS

Applicants gratefully note that all previous rejections have been withdrawn. Instant reply concerns the new objections and rejections.

The remarks below specify the changes made to the independent claims; however, the language of the dependent claims has been amended as necessary and as noted elsewhere in this reply.

Claim Objections

Claims 89 and 91 have been amended, changing the article from "The" to "A" in referring to the method of synthesis following Examiner's advice.

Claim Rejection – 35 USC § 101

Independent claims 51, 53, 74 and 75 have been amended to explicitly state that claimed compositions are not naturally occurring but synthesized, thus excluding the non-patentable subject matter.

Claim Rejection – 35 USC § 112 Second Paragraph

Current amendment of independent claims 51, 53, 74 and 75 clarifies that claimed oligonucleotide, besides comprising at least two CpG moieties, includes a nucleoside antimetabolite which is covalently linked within its structure.

Claim Rejection – 35 USC § 112 First Paragraph

Claims 51 and 53-88 stand rejected for failing to comply with the written description requirement set out in Section 112, paragraph 1 of the Patent Act:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or which it is nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out this invention.

The Office Action raises the following issues relevant to the above paragraph:

(a) The claims encompass genus of oligonucleotides, wherein the oligonucleotide itself comprises at least two CpG moieties and a nucleoside antimetabolite, but the written

description sets forth the "conjugate" comprising an oligonucleotide covalently linked to a nucleoside antimetabolite, wherein the oligonucleotide comprises at least two CpG moieties.

(b) The specification provides neither a representative number of oligonucleotides that encompass the genus nor does it provide a description of structural features that are common to the genus.

(c) The specification does not "clearly allow persons of ordinary skill in the art to recognize" that the inventor has invented what is claimed.

Applicants provide the explanation below to show that the current claims are fully supported by a written description in the specification as in the originally filed application. Moreover, based on the descriptions provided in the specification, a person of ordinary skill in the art could easily recognize what the inventor has invented and conclude that the inventor was in possession of the invention at the time of filing of the patent application.

The specification does provide a description of structural features that are common to the claimed genus

It is well settled that an Applicant need not limit the claims to that subject matter having *ipsis verbis* description. In *re Gosteli*, 872 F. 2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989) (stating that the written description requirement does not require an applicant to "describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (citations omitted)). Further, written description must be reviewed from the perspective of one of skilled in the art at the time the application is filed. *Wang Labs., Inc. v. Toshiba Corp.*, 993 F.2d 858, 863 (Fed. Cir. 1993).

The instant invention is concerned with the use of an oligonucleotide in the preferential killing of cancerous cells. The oligonucleotide of this invention suitable for the purpose of preferential killing of the cancerous cells has certain structural features. The oligonucleotides of the present invention comprise at least two CpG moieties and a covalently linked nucleoside antimetabolite, the covalent link being a key requirement. The oligonucleotide of the instant invention may be up to 50 nucleotides long in addition to having two or more CpG moieties.

The oligonucleotides of the instant invention comprise four distinct nucleotides. Similarly, there are a large number of nucleoside antimetabolites that can be covalently linked to the oligonucleotide. Thus the question is whether the specification provides enough written description so that persons of ordinary skill in the art could recognize that the inventors have invented a genus oligonucleotide with the capacity for preferentially killing cancerous cells, or in other words, if the specification conveys with reasonable clarity to those skilled in the art that,

as of the filing date sought, the inventors were in possession of the claimed invention. *Vas-Cath Inc.*, 935 F.2d at 1563-64 (Fed. Cir. 1991).

Whether the written description requirement is satisfied is a fact-based inquiry that will depend on the nature of the claimed invention, and the knowledge of the one skilled in the art at the time an invention is made and a patent application is filed. *Enzo,* 23 F.3d at 963.

A description of a genus may be achieved by means of recitation of representative number of species falling within the scope of the genus or by describing structural features common to the genus that constitute a substantial portion of the genus." See *University of California v. Eli Lilly and Co.,* 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997): "A description of a genus of cDNA, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." Subsequently in *Capon*, the Federal Circuit has elaborated on the written description requirement under first paragraph of 35 U.S.C. 112. *Capon v. Eshha*, 418 F.3d 1349, 1358 (Fed. Cir. 2005).

In *Capon*, the Federal Circuit held that "what is needed to support generic claims to biological subject matter depends on a variety of factors, such as existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter.

The invention in *Capon* involved "chimeric DNA that encodes single-chain chimeric protein for expression on the surface of the cells of the immune system, plus expression vectors and cells transformed by the chimeric DNA. The Federal Circuit held that the Board of Patent Appeals and Interference erred in holding that the written description requirement was not met because of failure to "reiterate the structure or formulas or chemical name of the nucleotide sequence of the claimed chimeric genus." The Federal Circuit held in *Capon* that the prior art contained "extensive knowledge of the nucleotide structures of the various immune-related segments of DNA," including "over 785 mouse antibody DNA light chains and 1,327 mouse antibody DNA heavy chains." Id. At 1355.

The present invention related to oligonucleotide with the potential for preferential killing of the cancerous cells is very similar to the invention in *Capon* and hence the specification required to support a generic claim should be assessed in terms of existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, and the predictability of the aspect at issue, along with the specification provided in the application as filed.

As enumerated below, the specification provides written description for synthesizing the oligonucleotides of the instant invention:

Paragraphs [0068] – [0070], [0075] and [0078] – [0082]: Written description of the oligonucleotide design and synthesis.

Paragraph [0064] and [0085]: Written description of the oligonucleotide backbone modification.

Paragraphs [0013] and [0017]: Written description of the linkers used in the synthesis of the oligonucleotides of the present invention.

Paragraphs [0076] and [0077]: Nucleoside antimetabolite useful in the present invention.

Paragraph [0086], [0087] and [0097 – 0117]: Written description for the attachment of nucleoside antimetabolite to the oligonucleotide.

Prior art teaching for attachment of nucleosidic and non-nucleosidic molecules to an oligonucleotide was well known at the time of filing of the instant patent application. The present invention teaches the introduction of the nucleoside in many ways: introduction of nucleoside antimetabolite at 5'- terminal of oligonucleotide, 3'- terminal of oligonucleotide, and at another specifiable position of the oligonucleotide during the automated oligonucleotide synthesis by solid support technology, which allows for the attachment of the phosphodiester linkage between the neighboring nucleoside in 5'-3' direction , 3'-5' direction, 5'-5' direction or 3'- 3' direction and a very flexible design scheme for synthesis of *specific*, requisite oligonucleotides.

Illustrations of these modes have been appropriately described in the specifications. In one embodiment the invention provides for the synthesis of an oligonucleotide where there is a covalent spacer between the nucleoside antimetabolite and oligonucleotide consisting of at least two CpG moieties. The nucleoside antimetabolite is attached in this case to oligonucleotide via a linker/ spacer.

At the time of filing of the instant patent application the field of nucleic acid chemistry was a matured field, and the results of the oligonucleotide synthesis were highly predictable. A number of techniques were routinely used in the synthesis of various forms of oligonucleotide as evidenced by the list of representative scientific publications attached to this response to office action (APPENDIX - A). Automated machines for the custom synthesis of oligonucelotides described in the present invention were easily accessible to the scientist in the field of nucleic acid chemistry in a large number of industrial and academic laboratories. A number of commercial suppliers of oligonucleotides were in existence at the time of filing of the present patent application for supplying the required oligonucleotides in a cost-effective way. For these reasons of technological advancements in the field of nucleic acid chemistry, a person skilled in the art of nucleic acid chemistry at the time of filing of the present application could have used

the teachings of the invention to synthesize any one of the oligonucleotides falling within the claimed genus.

To be certain, specification of the present invention provides a composition and process for synthesizing a "conjugate." Furthermore, the oligonucleotide of the present invention may be synthesized as a "conjugate" by covalently linking a nucleoside antimetabolite, either directly or through a spacer or linker, where the oligonucleotide contains at least two CpG moieties.

However, there exists a very large number of ways to incorporate a nucleoside antimetabolite within the claimed oligonucleotide described by: its structural features (e.g., at least two CpG moieties, a covalent link to nucleoside antimetabolite, and up to 50 nucleotides long), the linking modes (e.g., 5'- terminal, 3'- terminal, or within a specific position of the oligonucleotide), the attachment directions (e.g., 5'-3' , 3'-5' , 5'-5' or 3'- 3' direction) and the attachment methods (i.e., direct or via a spacer/linker).

Therefore, following *University of California* and *Capon* it is reasonable to conclude that support for the generic claim is provided by this exhaustive enumeration of possible species.

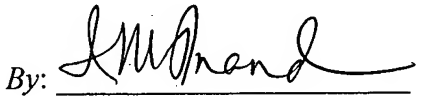
Adequacy of written description

The written description requirement for the claimed invention is met in this case based on the detailed description and teachings in the specification, when reviewed in the context of the knowledge of the prior art. Therefore, taken together, the Federal Circuit precedent addressing the issue, the discussion above and the examples provided show that the specification of the invention as filed had addressed in detail the various known modes and linkages, and generally provided support for the genus of synthetic oligonucleotides comprising at least two CpG moieties and a nucleoside antimetabolite.

Withdrawal of the rejection under 35 U.S.C. § 112 is therefore respectfully requested. Kindly contact the undersigned representative for any matter still outstanding to bring the application to allowance.

Dated: June 14, 2010

Respectfully submitted,
Suresh C. Srivastava, Satya P. Bajpai and Kwok-Hung Sit, Applicants

By: 

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APPENDIX - A

Prior Art References for Attachment Of Nucleosidic And Non-Nucleosidic Molecules to An Oligonucleotide

A number of prior art teaching for attachment of various molecules to oligonucleotides has been amply described. A number of such scientific publications are being cited here as example:

1. Antisense pro-drugs: 5'-ester oligodeoxynucleotides, N. N. Polushin and J. S. Cohen, **Nucl. Acids Res., 22, 5492-5496, 1994.**

The authors report introduction of lipophilic molecules at the 5'- terminal hydroxyl group of oligonucleotides in order to improve cellular uptake. It has been a general thought that oligonucleotides bearing a terminal lipophilic group attached through a biodegradable ester bond should be useful as antisense pro-drugs with improved cellular uptake. Since the synthesis of 5'-ester oligonucleotides is, somewhat problematic due to lability of the ester bond during aqueous ammonia treatment that is commonly used for the deprotection of synthetic oligonucleotides. The synthesis of 5'-palmitoyl oligodeoxynucleotides was accomplished in good yield by the use of a combination of base-labile tert-butylphenoxyacetyl amino protecting groups (t-BPA), the oxalyl-CPG anchor group, and ethanolamine (EA) as a deprotecting reagent".

The cited publication reveals that covalent attachment of a lipophilic molecule via a phosphodiester bond to the 5'- end of an oligonucleotide is possible and leads to better uptake of the oligonucleotide attached to lipophilic molecule. The authors rationalized that subsequent release of the lipophilic molecule in vivo makes the oligonucleotide (without the lipophilic portion of the oligonucleotide) available for exerting its pre designed biological properties. The authors in the cited publication utilized palmitoyl group for covalent attachment to oligonucleotide as a phosphodiester bond via phosphoramidite chemistry and solid phase oligonucleotide synthesis.

2. Biotinyl and phosphotyrosinyl phosphoramidite derivatives useful in the incorporation of multiple reporter groups on synthetic oligonucleotides; Konrad Misiura, Ian Durrant, Michael R. Evans and Michael J. Gait. Nucl. Acids Res., 15, 4345-4354, 1990.

The article reports attachment of a biochemical reporter molecule to an oligonucleotide. Example of prior art reports attachment of biochemical molecules responsible for biochemical events or site of hybridization of an predesigned oligonucleotide has been carried out. Thus biotin and phosphotyrosine were attached to oligonucleotide during solid phase method via phosphoramidite chemistry as phosphodiester bond to oligonucleotide.

The authors describe non-nucleosidic phosphoramidite linker units suitable for use on commercial DNA synthesizers and both; biotin and a new reporter group, phosphotyrosine, were directly incorporated at multiple sites on synthetic oligonucleotides. The units were based on a 3-carbon glycerol backbone where the reporter group was attached to the 2-O-position through a 3-aminopropyl spacer. 17-mer oligonucleotides were synthesized which carried 1, 2, 4 or 8 biotinyl units or 1, 2, 4 or 8 phosphotyrosinyl units respectively at the 5'- end and were used for the detection of DNA on nitrocellulose filters by hybridization. Subsequent incubation was carried out on filters with a monoclonal antibody to the reporter group followed by secondary detection using enhanced chemiluminescence (ECL). This resulted in amplification of signal strengths as the number of reporter groups was increased. The authors quantitated the results by use of a charge couple device (CCD) camera. It was noted that spacing of biotin moieties by thymidyl residues resulted in further improvements in signal strengths, whereas similar spacing of phosphotyrosinyl units did not improve the signal strength.

3. Attachment of a propyl amino group at 3'- end of an oligonucleotide. Synthesis of phosphoryl amine terminal oligonucleotidees, Joseph G. Zendegui, Karen M. Vasquez, John H. Tinsley, Donald J. Kessler and Michael E. Hogan, Nucl. Acids Res., Vol. 20, 307-314, 1992.

The article reports *in vivo* stability and kinetics of absorption and disposition of 3' phosphopropyl amine oligonucleotides. This article provides a method of synthesis of 3'- propyl amino function attached to G- rich oligonucleotides which are useful for triplex formation. The attachment of the propyl amino group to oligonucleotide was achieved via phosphoramidite solid phase chemistry and the group is attached via a phosphodiester bond between the target oligonucleotide and propyl amine functionality.

The abstract of the article is cited here.

The authors proposed to develop oligonucleotide derivatives as therapeutic agents and in order to understand their pharmacokinetic behavior. They reported in *vivo* disposition and stability of a prototype of such compounds. The oligonucleotide which was G-rich 38 base 3' phosphopropyl amine oligonucleotide designated as TFO-1, was found to clear from the circulation with a half-life of approximately 10 minutes, displaying distribution kinetics consistent with a two compartment model. TFO-1 was found to readily absorb into circulation from the peritoneal cavity. The authors examined all tissues except brain and found accumulation of the compound reaching concentrations calculated to be in the micro molar range. It was found that TFO-1 was stable in circulation and in tissues in that a large fraction of intact material was detected 8 hours after injection, and assessed by gel electrophoresis. The authors observed that approximately 20–30% of the injected dose was excreted in the urine over an 8 hour period. The authors concluded

that G-rich oligonucleotides, minimally modified at the 3' end, are relatively stable *in vivo* and have distribution kinetics favorable to use as therapeutic agents.

4. Oligonucleotide labeling methods. Direct labeling of oligonucleotides employing a novel, non-nucleosidic 2-aminobutyl-1,3-propanediol backbone, P. S. Nelson, M. Kent, and S. Muthini; **Nucl. Acids Res., Vol. 20, 6253-6259, 1992.**

This article reports process for introduction of single or multiple labels comprised of several fluorescent molecules and terminal amino functions to oligonucleotides covalently as a phosphodiester. The process of synthesis involved phosphoramidite method of oligonucleotide synthesis on solid supports. The terminal amino group of linker was functionalized with various reporter molecules such as fluoresceine, acridine dyes, biotin, LC ,biotin and primary amine functions.

The article outlined synthesis of a number of cyanoethyl -phosphoramidite and nucleoside modifies attached to solid supports (CPG) which were prepared from a unique 2-aminobutyl-1,3-propanediol backbone. The reagents have been used to directly label oligonucleotides with fluorescein, acridine, and biotin via automated DNA synthesis. The reagent 2-aminobutyl-1,3-propanediol backbone allowed for labeling at any position (5', internal, and 3') during solid phase oligonucleotide synthesis. Multiple labels were achieved by repetitive coupling cycles. Furthermore, the 3-carbon atom internucleotide phosphate distance is retained when inserted internally. Using this method, individual oligonucleotides possessing two and three different reporter molecules were prepared.

5. Inhibition of simian virus 40 DNA replication in CV-1 cells by an oligodeoxynucleotide covalently linked to an intercalating agent. F Birg, D Praseuth, A Zerial, N T Thuong, U Asseline, T Le Doan, and C Hélène, **Nucl. Acids Res., 18, 2901-2908, 1990.**

The article reports linking of oligodeoxy nucleotides to intercalating agents. The authors covalently linked an octnucleotide consisting of thymidine via its 3'-end to an acridine derivative and noted that this oligonucleotide inhibited the cytopathic effect of Simian Virus SV40 on CV-1 cells in culture. The authors further observed that this effect was virus-specific and did not arise as a result of oligonucleotide degradation by nucleases. The authors further attached a photoactive probe covalently at the 5'-end of the oligonucleotide-acridine conjugate. Subsequently this oligonucleotide was UV-irradiated, resulting in photocrosslinking at the A rich region of the virus origin of replication. The authors noted that a local triple helix can form at moderate salt concentrations with two octathymidylate-acridine conjugates bound to the octaadenylate sequence and the octathymidylate-acridine conjugate can bind to the major groove of duplex DNA forming a local triple helix. The authors discussed different mechanisms for the inhibition of viral DNA replication.

6. The Mox/Suc Precursor Strategies: Robust Ways to Construct Functionalized Oligonucleotides , N. Polushin , **Nucleoside Nucleotide & Nucleic Acids, 20 (4-7), 973-976 (2001).**

This article describes a robust method of synthesis of multiple functionalized oligonucleoitides. Various phosphoramidites bearing MOX or SUC residues were synthesized . Defined sequence oligonucleotides were synthesized utilizing such phosphoraimidites via solid phase automated oligonucleotide synthesis. Thus one or more methoxy oxalamide (MOX) or succinimido (SUC) reactive group can be utilized for construction of functionalized oligonucleotides. The MOX and SUC are amine reactive functions .A nucleoside or non nucleoside phosphoramidite was synthesized bearing MOX and SUC functions. The MOX and SUC group bearing phosphoramidites are then incorporated in a defined sequence oligonucleotide. These functions were used to introduce amine containing molecule. It was demonstrated that up to 16 units of imidazole residues can be attached to an oligonucleotide.

7. Synthesis And Evaluation of Oligodeoxyribonucleotides Containing An Aryl(trifluoromethyl)diazirine Moiety As The Cross-Linking Probe: Photoaffinity Labeling of Mammalian DNA Polymerase beta. T. Yamaguchi, K. Suyama, K. Narita, S. Kohgo, A. Tomikawa and M. Saneyoshi, Nucl. Acids Res., 3672-3680, 1997

The article describes synthesis of a photolabile nucleoside, 2'-deoxy- E -5-[4-(3-trifluoromethyl-3 H-diazirin-3-yl)styryl]uridine and the corresponding protected phosphoramidite derivatives . The later was incorporated into defined sequence DNA oligomers through solid-phase DNA synthesis. The photoreactive component (trifluoromethyldiazirinyl)stylyl of the nucleoside was found to be sufficiently stable for automated DNA synthesis. Further the photolabile moiety was found to be stable at 60 degrees C in aqueous solution under the annealing conditions for duplex formation with complementary strands. An oligonucleotide, (dT) 15mer analog bearing the photolabile residue was activated/decomposed by near-UV irradiation and cross linked with recombinant rat DNA polymerase beta. It was shown that synthesis of photolabile oligodeoxyribonucleotides may be useful for such studies.

8. A new method of synthesis of fluorescently labeled oligonucleotides and their application in DNA sequencing, W. T. Mickiewicz, G. Gorger, R. Rosh, A. Zebrowska, M. Markiewicz, M. Klotz, M. Hinz, P. Godzina, and H. Seliger, **Nucleic acids Research, 3611-3620, 2001.**

The article reports chemical synthesis of oligodeoxynucleotides bearing reporter functional groups at nucleoside base residues at the 3'-end of oligonucleotides. The reporter group were chosen to be various types of fluorescent dyes. The attachment to oligonucleotide was carried out on automated DNA synthesizer. The labeled oligonucleotides having 3'- end fluorescent group thus obtained were found to be very useful as primers for automated DNA sequencing.

9. Oligodeoxynucleotides containing 2'-O-modified adenosine: synthesis and effects on stability of DNA: RNA duplexes., Lesnik EA, Guinosso, C.J, Kawasaki AM, Sasmor H, Zounes M, Cummins LL, Ecker DJ, Cook PD, Freier SM. **Biochemistry. 1993, Aug 3;32(30):7832-8.**

The paper reports hybridization thermodynamics for various oligonucleotide sequences containing 2'-fluoro dA, 2'-O-methyl A, 2'-O-ethyl A, 2'-O-propyl A, 2'-O-butyl A, 2'-O-pentyl A, 2'-O-nonyl A, 2'-O-allyl A, and 2'-O-benzyl A substituting deoxyadenosine. It was observed that for six sequences containing a few 2'-modified adenosines in relation to unmodified deoxynucleotides, the average delta TM per substitution ranged from +1.3 degrees C for 2'-fluoro dA to -2.0 degrees C for 2'-O-nonyl A. In case where adenosine was substituted with various 2'-O-alkyl substituents, the average delta TM per substitution changes with the bulk and size of the alkyl substituent, and the order of stability of duplex was found to be; 2'-O-methyl A > 2'-O-ethyl A > 2'-O-propyl A > 2'-O-butyl A > 2'-O-pentyl A > 2'-O-nonyl A. The authors extended the study to 2'-fluoro dA, 2'-O-allyl A, and 2'-O-benzyl Adenosine (A)

10. Oligonucleotide Analogs With Terminal 3'-3'- OR 5'-5' Internucleotide Linkage, Inventors, H. Rosch, A. Frohlich, J. Flavio Ramallo-Ortigao, M. Montenarh, H. Selliger, Assignee Hoechst Aktiengesellschaft, **US Patent 5,750,669, May 12, 1998**

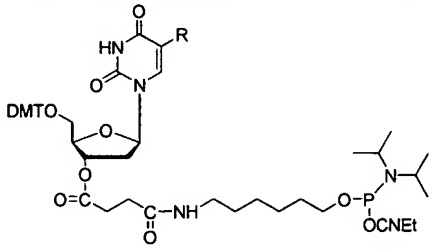
This patent describes synthesis of therapeutically useful oligonucleotides which have terminal 3'-3' or 5'-5' phosphodiester linkage between the terminal base and the preceding base of the oligonucleotide. It was shown that such terminal linkage allow stability towards endonucleases and increase cellular stability of the oligonucleotides. The patent discloses methods of synthesis and incorporation of such linkages Further the patent discloses serum stability data of such oligonucleotides.

11. 3'-Modified oligonucleotides by reverse DNA synthesis; Christopher D. Claeboe, Rong Gao, and Sidney M. Hecht, **Nucl. Acids Res., 31,19, 5685-5691 (2003).**

The paper presents a study of DNA synthesis in the reverse direction, i.e., 5'- 3'- direction. The authors exploited this strategy to significant extent for synthesis of a number of synthetic DNA's. Although the monomer synthons were previously made in order to attach alternating 3'-3'- and 5'-5'-linked nucleosides as part of study to evaluate deoxy oligonucleotides for antisense therapeutics. The methodology required synthesis of nucleoside 5'-phosphoramidites and subsequently oligonucleotides were synthesized. The authors utilized reverse oligonucleotide synthesis for the facile synthesis of 3'-modified DNAs and attached a phosphoramidite derived from tyrosine. The derived oligonucleotide was shown to have chromatographic and electrophoretic properties identical with the modified oligonucleotide resulting from the proteinase K digestion of the vaccinia topoisomerase I–DNA covalent complex.

12. Attachment of carboxy generating ligand to oligonucleotide via phosporamidite method of oligonucleotide synthesis.

A carboxy generating reagent which has a phosphoramidite group and a terminal ester function at the 3'- hydroxyl group of 5'- hydroxyl protected thymidine. The structure is shown below, and was developed by ChemGenes Corp. As catalog # CLP-2244. After the synthesis of a defined sequence oliogonucleotide, this linker is coupled to 5'- hydroxyl group of oligonucleotide terminal during solid phase oligonucleotide synthesis. The final oligonucleotide is deprotected and during deprotection the ester function gets smoothly hydrlolyzed releasing free carboxyl group at the 5'- end of an oligonucleotide. The free carboxylic function can then be utilized to attach a variety of terminal amine containing molecules with the formation of amide linkage under appropriate pH conditions.



{(Thymidine succinyl hexamide CEP – phosphoramidites ;CLP -2244)}

The phosphoramidite is a useful ligand for coupling to oligonucleotide of choice at the terminal via solid phase phosphoramidite synthesis. After deprotection the oligonucleotide possesses a free carboxyl group at the terminal of oligonucleotide, while thymidine is cleaved off and washed out. The carboxyl function is activated appropriately , followed by coupling with a molecule bearing free amine.

13. Disclosure of Modified Nucleoside bases in specifications. F. Seela & H. Driller, Nucl. Acids Res.,13, 911,1986; F.Seela & H. Winter, **Helv. Chim. Acta,77, 597 (1994);** F. Seela & H. Winter, **Helv. Chim. Acta., 81, 2244(1998)**

Modified nucleoside bases include 5- methyl cytosine, 5- azacytosine, 5- halogen substituted (F, Cl, Br, I) , 5- alkyl subsuituted uracil or cytosine , C-5 propyne uracil, C-5 propyne cytosiner. Purine modified bases, 7- deazaadenine, 7- deazaguanine, -7-iodo-7-deazaadenine, 7- deaza-7-iodo-guanine, 7- propyn-7- deaza-adenine, 7- propyn-7- deazaguanine are envisage to replace natural bases within the sequences. Such nucleosides are well known in the art. A list of references [1-3] are being provided known in the art prior to the current invention date, which detail incorporation of many of such modified nucleosides in the defined sequence synthetic DNA .

14. 5'- Terminal, internal or 3'- terminal amino introducing reagents: P.S. Nelson, R.S. Gold, R. Leon. **Nucl. Acids Res., 17, 7179, 1989**

A number of reagents were developed by different research groups, in order to introduce a functional group such as a primary amine into a synthetic oligodeoxynucleotide or oligonucleotide. The primary amine function, once introduced at a position of choice into an oligonucleotide then could be functionalized with variety of labeling reagents. Covalently linked chromophores were attached to an oligonucleotide in a synthetic DNA or RNA of interest. .

15. 3'- Amino modifier: Lagos- Quintana et. Al., Current Biology, 12, 735-739, 2002

Attachment of terminal amino group to an oligonucleotide is generally achieved by use of 3'- amino modifier C-6 long chain alkyl amino (Icaa) Controlled pore glass (CPG). One such 3'- amino modifier ligand attached to a solid support was introduced by ChemGenes Corporation, known as N-1004. This has been in use for a very long period of time. Automated oligonucleotide synthesis on this support followed by deprotection of protecting groups leads to an oligonucleotide which has a terminal free amine (-NH2 group) at the 3'- terminal. This modification has been found to a very useful addition for synthesis of 3'- amino linked DNA-RNA chimeras and for micro RNA cloning;

16. Butane diol spacer:
Butanediol spacer as phosphoramidite on automated DNA synthesizer, has been incorporated in oligonucleotides consisting of 2',5'- adenosine, combined with an oligonucleotide of choice. The chimeras were found to effectively catalyze selective cleavage of RNA in human cells.
R.K Maitra, G. Li., W. Xiao, B. Dong, P.F. Torrence, R.H. Silverman, **J. Biol. Chem., 270, 15071, 1995;** K. Lesiak, S. Khamnei, and P.F. Torrence, **Bioconjugate Chemistry, 4, 467, 1993.**

17. 5'- Thiol Modifier C-6 Phosphoramidites :

Terminal thiol/sulfhydryl function (-SH) containing oligonucleotides are useful for conjugation of various macromolecules via disulfide linkage. S-trityl modified C-6 phosphoramidite has been utilized for this purpose. After oligonucleotide synthesis, following by coupling with this class class of phosphoramidite on an automated DNA synthesizer, the oligonucleotide contains a S-Trityl at the 5'- terminal base intervened by a C-6 spacer. The S- Trityl group is subsequently cleaved to generate a free –SH group. The free –SH (sulfhydryl) group which can then be

coupled with other molecules bearing a sulfide group (-SH) to form a disulfide linkage between oligonucleotide and the molecule of interest via a hexyl spacer.

Another terminal sulfhydryl generating reagent, 5'- Thiol- C-6 disulfide phosphoramidite has been utilized to introduce various molecules of interest to an oligonucleotide coupled via disulfide linkage. The phosphoramidite is first introduced at the 5'- position of an oligonucleotide via automated oligonucleotide synthesis methodology. Subsequently the disulfide function is cleaved to generate free sulfhydryl (–SH) group in solution. The free – sulfhydryl group is subsequently coupled to a molecule of interest bearing a free SH group and formation of a disulfide bond between oligonucleotide and other molecule of interest .(B. A.Connolly, P. Rider, Nucl. Acids Res., 12, 4485, 1985; N.D. Sinha, R.M. Cook, Nucl. Acids Res., 16, 2659, 1988; S. Sharma, K.C. Gupta, Nucl. Acids Res., 14, 4404, 1989; A. Kumar, S. Advani, H. Dawar, G.P. Talwar, Nucl. Acids Res., 19, 4561, 1991)

18. Biotin Phosphoramidites : (structures page 80):

Biologically active molecules such as biotin appropriately functionalized and converted to phosphoramidites, followed by incorporation into oligonucleotide via automated DNA synthesis (Uwe Pieles, Brian Sproat and Gabor M. Lamm, Nucl. Acids Res., 18, 4355, 1990; Biotin BB Phosphoramidites, ChemGenes catalog items, cat # CLP-1502 & N-9162-05).

19. Branching Phosphoramidites:

The branching phosphoramidites allow branching sites into an oligonucleotide via automated DNA synthesis directly. Branched oligonucleotides are synthesized from the glycerol backbone containing phosphoramidite, which possess two protected hydroxyl groups for chain elongation. Branched oligonucleotides have been used effectively for signal amplification by introducing network of labels or probing sites (Horn et al., 1989; Chang et al., 1991; Foldesi et al., 1991).

20. Site specific functionalization of oligonucleotides for attaching two different reporter groups; Sudhir Agrawal and Paul C. Zamecnik, Nucleic Acids Research, pp 5419-5423, 1990.

The article reports synthesis of an oligonucleotide functionalized with two different reporter groups at specific internucleotide linkages. The two functional groups selected were; phosphorothioate and the second was amidate group carrying a terminal amine function. Under the condition for attacjment of thiol specific reagent , the amine function was unreactive. Thus after attachment of thiol specific reagent the amino group was functionalized with flureseine isothiocyanate. The synthetic cycle for introducing the modified internucleotide linkages at

specific sites were carried out by using an automated DNA synthesizer. The authors used two reporter groups successfully to a oligonucleotide and utilized solid phase synthesis technology.

21. Synthesis, hybridization properties and antiviral activity of lipid-oligodeoxynucleotide conjugates. Regan G. Shea, James C. Marsters and Norbert Bischofberger, Nucleic Acids Research, pp 3777-3783, 1990.

This article reports attachment of lipids to oligonucleotides. An appropriate lipid was functionalized as H- phosphonate and added to oligonucleotide via H- phosphonate methodology of oligonucleotide synthesis. Thus 1, 2-di-O-hexadecyl-*rac*-glycero-3-H-phosphonate triethylammonium salt was coupled to the 5' terminus of oligodeoxynucleotides via hydrogen phosphonate solid support DNA synthesis methodology. The oligonucleotide conjugated with a single lipid unit was subsequently hybridized with a complementary oligonucleotide and was found to have lower melting temperatures than the corresponding unmodified duplex, but duplexes bearing lipids at each 5' end had higher T_ms. The authors conducted uptake studies on lipid conjugated oligonucleotides in L929 cells and observed that 8–10 times more lipid-DNA became cell-associated than did unmodified DNA. The authors found that unmodified antisense diesters were inactive in a VSV antiviral assay in L929 cells (at up to 200 μM). It was observed that attachment of a lipid to the oligomer led to a >90% at 150 μM (>80% at 100 μM) reduction in viral protein synthesis. Lipid conjugated oligonucleotides were also converted to phosphorothioate derivatives and found to have reduced viral protein synthesis by 20 – 30% at 100 μM in the VSV assay. The authors report that the lipid-DNA compounds were not toxic to the cells at up to 100 μM.

22. Preparation of a novel psoralen containing deoxyadenosine building block for the facile solid phase synthesis of psoralen-modified oligonucleotides for a sequence specific crosslink to a given target sequence. U. Pieles, B.S. Sproat, P. Neuner and F. Cramer, Nucleic Acids Research, pp 8967-8978, 1989.

The authors synthesized psoralen derivative as phosphoramidites. Thus 4,5',8-Trimethylpsoralen was attached to the C8- position of deoxyadenosine via a sulfur atom and a five carbon atom linker. The modified deoxyadenosine was then converted to a protected phosphoramidite and then attached to oligonucleotide via solid phase oligodeoxyribonucleotide synthesis. Oligonucleotide attached to psoralen derivatives were subsequently studied for their cross linking properties. It was found that the efficiency of the photoreaction of a psoralen-modified oligonucleotide to a complementary matrix strand reached more than 90% within a 1hour irradiation time at a wavelength of 345 nm. 23. Synthesis and use of labeled nucleoside phosphoramidite building blocks bearing a reporter group: biotinyl, dinitrophenyl, pyrenyl and dansyl; **A. Roget, H. Bazin and R. Teoule. Nucleic Acids Research, pp 7643-7651, 1989.**

The authors report synthesis of protected nucleoside phosphoramidites bearing various markers such as biotinyl, dinitrophenyl, dansyl and pyrenyl groups are reported. These labeled deoxynucleosides phosphoramidites were used for solid phase oligonucleotide synthesis in the same way than the usual protected phosphoramidites without any change in the synthetic cycle and the deprotection step. The authors used the labeled building blocks in conjunction with the labile base protected phosphoramidites ('PAC phosphoramidites') which allowed mild ammonia deprotection, especially recommended for the dinitrophenyl-labeled oligonucleotides. The authors reported multiple labeling (i.e. 10 to 20 biotins) efficiently on the same oligonucleotide, resulting in an increase of sensitivity. The polylabelled oligonucleotides were analyzed appropriately and resulted in increased signal and low background coloration for *in situ* hybridization. 4'-Thio-oligo-ß-D-ribonucleotides: synthesis of ß-4'-thio-oligouridylates, nuclease resistance, base pairing properties, and interaction with HIV-1 reverse transcriptase.

24. 4'-Thio-oligo-ß-D-ribonucleotides: Synthesis of ß-4'-thio-oligouridylates, nuclease resistance, base pairing properties, and interaction with HIV-1 reverse transcriptase. Laurent Bellon, Jean-Louis Barascut, Georges Maury, Gilles Divita[1], **Roger Goody[1] and Jean-Louis Imbach, Nucleic Acids Research, 1587-1593, 1993.**

The authors reported synthesis and study of properties of a new series of modified oligonucleotides, namely 4'-thio-oligo-ß-D-ribonucleotides (4'-S-RNA). Homo-oligonucleotides of this class (4'-SU_6 and 4'-SU_{12}). The thionucleoside phosphoramidites were first prepared using known thionucleosides. Subsequently the phosphoramidite obtained were converted into oligonucleotide (4'-SU_6 and 4'-SU_{12}) by solid phase oligonucleotide synthesis methodology. The authors compared the substrate properties of 4'-SU_6 and its natural analog U_6 with respect to four nucleases and found that the former is much more resistant than the latter. The authors concluded that the nuclease resistance properties and the high Tm values for 4'-SU_6 hybridized with Poly(A) are suitable to develop to good candidates for potential antisense effects. The authors evaluated the oligonucleotides 4'-SU_6 and 4'-SU_{12} as non sequence specific inhibitors of HIV-1 reverse transcriptase.

25. Synthesis and antibody-mediated detection of oligonucleotides containing multiple 2,4-dinitrophenyl reporter groups, John Grzybowski, David W. Will, R.E. Randall, Clive A. Smith and Tom Brown, Nucleic Acids Research, Received March 10, 1993. Revised September 23, 1993. Accepted September 23, 1993.

The authors developed a number of non-nucleoside-based 2,4-dinitrophenyl (DNP) phosphoramidites and used them in the multiple labeling of oligonucleotides utilizing solid-phase based oligonucleotide synthesis. The authors varied length of spacer arm between the DNP label and the oligonucleotide phosphate backbone. Further the number of DNP units were varied to determine the optimum conditions for anti-DNP antibody binding. The authors

observed that using enzyme-linked colorimetric detection techniques the sensitivity was equivalent to that obtainable using biotinylated oligonucleotides.

26. Incorporation by chemical synthesis and characterization of deoxyribosylformylamine into DNA, Andrè Guy, Anne-Marie Duplaa, Jacques Ulrich and Robert Tèoule, Nucleic Acids Research, 5815-5820, 1991

The article reports characterization of 2-deoxyribosylformylamine as a major oxidative DNA damage product which occurs upon the action of ionizing radiation on DNA. The authors synthesized appropriately protected 2-deoxyribosylformylamine phosphoramidite and used this derivative in conjunction with previously reported alkali labile base protected phosphoramidites('PAC phosphoramidites') for the preparation of oligodeoxyribonucleotides containing this lesion. The authors carried out final deprotection of the oligonucleotides under mild alkaline conditions to preserve the integrity of the fragile defect. The authors confirmed presence of formylamino deoxyribosyl residue by FAB mass spectrometry sequencing. The authors used the oligonucleotides bearing deoxyribosyl formylamine as templates for studying *in vitro* replication and found them to direct insertion of guanine or induce a deletion opposite the lesion.